bio-protocol Preprint

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Sampling

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An abbreviated version of this protocol was published in eLIFE in May 2019

Independent amylase gene copy number bursts correlate with dietary preferences in mammals

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Detailed protocol

Saliva was collected using Oasis Diagnostics Micro SAL or Super SAL oral fluid/saliva collection devices, depending on the size of the oral cavity, per manufacturer's instructions. Micro SAL devices were used to collect from cats and giant African pouched rats. Super SAL devices were used for all other species. Animals were not allowed to eat or drink for 10 minutes prior to the collection to ensure the oral cavity was free of food and other debris. For saliva collection using the Super Sal device, animals were gently restrained and the collector tip was placed under the tongue for up to three minutes, or until the sample volume adequacy indicator changed from white to red. For saliva collection using the Micro SAL device from cats and giant African pouched rats, the collection occurred while the animal was anesthetized for another clinical procedure. The collector tip was placed under the tongue for the duration of the procedure. The devices were stored at -20^0 F before shipping on dry ice. DNA samples were provided by collaborators named in supplementary data. Methods of DNA collection include buccal swabbing of the oral cavity, blood samples, and frozen tissue samples (museums). If more precise information is needed, please email: Petar Pajic directly at petarpaj@buffalo.edu

Collection of saliva of specific samples

Saliva samples and buccal swabs from deer mice (*Peromyscus spp.*) were provided by Danielle Garneau (SUNY Plattsburgh). Mice were trapped in the wild by *Sherman* live traps *(Garneau et al., 2012)*. After restraint by scruffing behind the neck, a glass capillary tube was introduced to the animal's mouth and was moved about the lower lip and cheeks to collect saliva. The tube was introduced at an angle such that gravity would help draw down the sample into the tube. The capillary tube was placed in an Eppendorf tube and a pipet pump was used to force air to drive the rest of the sample from the capillary tube into the Eppendorf tube for storage at -20°C and shipment on dry ice.

Saliva from house mice (laboratory strain C57BL10/SNJ) was kindly provided by Jill Kramer (University at Buffalo) using a collection procedure as previously described (Kiripolsky et al., 2017).

Saliva from woodrats was kindly provided by Michelle Skopec (Weber State University). To collect saliva, woodrats were scruffed and Micro-Sal collection (Oasis Diagnostics, Vancouver, WA) devices were placed in their mouths. The woodrats were allowed to chew on the absorbent sponge part of the device and, then, their tongues and cheeks were swabbed to retrieve residual saliva. Collection devices were centrifuged and saliva samples were stored at -20°C before shipment on dry ice.

Saliva from Long Evans hooded rats was kindly provided by Ann-Marie Torregrossa (University at Buffalo). As described previously(Martin et al., 2018; Torregrossa et al., 2014), rats were conditioned to salivate when a pipette was inserted into the mouth and saliva was collected. Approximately 50 µl saliva was retrieved by suction from below and around the tongue where it pools naturally.

Saliva from dogs, cows, sheep, goats, horses, pigs, and giant African pouched rats was provided by Erin Daugherity and Luce E. Guanzini (Cornell University). Animals were not allowed to eat or drink prior to the collection to ensure the oral cavity was free of food and other debris. Saliva from giant African pouched rats was collected opportunistically while animals were anesthetized for an unrelated clinical procedure. The collection was performed using a commercially available device (Micro-Sal, Oasis Diagnostics). Large animals were gently restrained and a larger collection device (Super-Sal, Oasis Diagnostics) was placed under the tongue for up to three minutes, or until fully soaked. Devices were stored at -20 °C before shipping on dry ice.

Saliva from female wild boars and castrated domestic pigs were provided by Anja Globig (Friedrich-Loeffler-Institut, Insel Riems - Greifswald, Germany). For the collection of saliva a commercial collection device, consisting of an absorbent cotton swab in a tube, was used (Salivette® , Sarstedt, Nümbrecht, Germany). The swab was inserted in the animal's mouth and fixated with a forceps until it was drenched with saliva. After placing the swab back in the tube, saliva was extracted by centrifugation. Samples were lyophilized before international shipping.

Saliva from wolves was kindly provided by Karen Davis (Wolf Park, Battle Town, IN). The wolves housed in this facility are well socialized, which allowed saliva collection by inserting Super-Sal (Oasis Diagnostics) devices into the mouths of adult wolves willing to participate. Swabs were kept in the animals' mouths as long as they would tolerate it or until fully soaked. Samples from juvenile wolves could be collected while they were resting by inserting the swabs into their mouths. Samples were stored at-20°C before shipment on dry ice.

Saliva from dogs was kindly provided by Barbara McCabe (Buffalo, NY). Samples were obtained from diverse breeds of dogs including Boxers, Pitbulls

Saliva from dogs was kindly provided by Barbara McCabe (Buffalo, NY). Samples were obtained from diverse breeds of dogs including Boxers, Pitbulls, Golden Retrievers, and Labradors, along with several mixed breeds (see Table S1 for details). Super-Sal devices (Oasis Diagnostics) were placed in the mouth of dogs for 1-5 minutes, or until swab was damp. The swabs were stored at -20°C until transfer to our laboratory.

Saliva from Ring-tailed Lemur samples was kindly provided by Erin Ehmke (Duke Lemur Center). Samples were collected using commercially available absorbent strips (SalivaBio Children's Swabs, Salimetrics, Carlsbad, CA). Saliva-soaked swabs were immediately centrifuged and the collected saliva was frozen at -80°C and shipped on dry ice.

Saliva from humans was collected by passive drooling following the protocol approved by the University at Buffalo Human Subjects IRB board (study # 030-505616). Informed consent was obtained from all human participants. Saliva from chimpanzees and gorillas was collected in a noninvasive manner following the protocol approved by the University at Buffalo IACUC committee (IACUC ID# AR201800024). Chimpanzees were trained by the caretaker to voluntaril expectorate into a plastic cup. Gorilla (Western lowland gorilla) saliva was collected by the animal caretakers with a soft disposable plastic Pasteur pipette (VWR, Radnor, PA) from individuals who were previously trained to open their mouth upon request. Saliva from Rhesus macaques was provided by the Southwest National Primate Research Center, San Antonio, TX, and by the Yerkes National Primate Research Center, Atlanta, GA. All samples were immediately transferred into a polypropylene tube chilled on ice. Aliquots were stored at -80°C and shipped on dry ice.

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1. Pajic, P. , Ruhl, S. and Gokcumen, O. (2022). Sampling. Bio-protocol Preprint. 10.21769/p1529.
2. Pajic, P., Pavlidis, P., Dean, K., Neznanova, L., Romano, R., Garneau, D., Daugherity, E., Globig, A., Ruhl, S. and Gokcumen, O.(2019). Independent amylase gene copy number bursts correlate with dietary preferences in mammals. eLIFE. DOI: 10.7554/eLife.44628